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Detectors: Mass Spectrometry

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Introduction

Liquid chromatography (LC) can often separate complex mixtures but simple detectors (e.g. ultraviolet-visible UV/Vis) do not allow identification of the individual components. Comparison of retention data and spiking with known standards is normally required to provide evidence of composition but this may lead to erroneous results as absolute identification is not possible. Development of diode array detection has somewhat alleviated the problem but not removed it entirely. Absorbance requires the presence of a chromophore in the molecule and, as such, UV/vis spectra do not enable absolute identification but are frequently used to confirm identity through comparison of recorded spectra with reference spectra.

Mass spectrometry (MS) provides a unique means of determining the presence of a compound in a mixture by producing a mass spectrum which will aid or confirm its identification. The relative molar mass (RMM) and/or structurally important information may also be obtained from the mass spectrum.

The combination of a separation technique with MS provides a powerful instrumental method for the analytical scientist. Modern gas chromatography–mass spectrometry (GC-MS) instrumentation, having overcome the obstacles associated with coupling them to each other, has matured into an easy-to-use benchtop technique. The interfacing of high performance liquid chromatography (HPLC) with a mass spectrometric detector (LC-MS) poses many problems, not least the different sample requirements of the respective instruments, i.e. liquid and vapour. The purpose of this article is to describe those interfaces that are most routinely used in LC-MS applications and, as such, will cover aspects of ionization methods and, to a lesser extent, mass analysers.

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Background

The combination of HPLC and MS can be used as an offline technique, that is, fractions are collected and then a mass spectrum of each obtained. Much greater sensitivity, however, may be achieved by having an online interface, but this is much more difficult to achieve than with GC. The vapour flow in HPLC is much greater than in GC and there may be problems with electrical breakdown in high voltage instruments. HPLC may be operated in either normal or reversed-phase modes and the mobile-phase composition may be either isocratic or gradient. Different-sized columns are available (analytical, microbore and capillary), leading to a wide range of operational flow rates. The various possible configurations complicate the interfacing to MS.

The development of LC-MS has a history of more than 20 years and many interfaces have been reported, although only a small number have become commercially successful. Each method has its advantages and disadvantages (i.e. there is no universal interface). All facilitate the transition of analyte from solution into the gas phase with either simultaneous or sequential ionization. Those interfaces that have stood the test of time and are (or have been)

commercially available have relied on a particular ionization method and this presented limitations to the range of compounds that could be handled. Until the early 1980s, the mainstay ionization techniques were electron ionization (EI) and chemical ionization (CI), both of which required the sample to be in the vapour state. Development of LC-MS was slow, due to problems of matching vacuum requirements with liquid flow. Early interfaces utilized direct liquid introduction, usually after splitting the LC eluate. Thermal evaporation of the fraction of the liquid taken into the MS was followed by either EI or CI, with the reagent ions being generated from the solvent in the case of CI.

Of the many interfaces that have been reported, those most commonly employed in present applications are based on the following: particle beam (monodisperse aerosol generator interface for chromatography or MAGIC), continuous flow–fast atom bombardment (CF-FAB), thermospray and atmospheric pressure ionization.

Ion Formation

Particle Beam

The original particle beam interface was introduced by Willoughby and Browner in the mid-1980s using the acronym MAGIC. It relies on the nebulization of the chromatographic eluent followed by desolvation and then ionization of the resultant microparticles. A schematic of a typical particle beam interface is shown in **Figure 1**.

The initial nebulization of the eluent is accomplished with the aid of a dispersion gas (usually helium); thus a fine and homogeneous aerosol can be generated from mobile-phase flow rates ranging from 0.1 to 2.0 mL min^{-1}. Several designs of nebulizer are available, some utilizing heat or ultrasound in addition to a gas, to create the aerosol. The resultant mixture of gas and solvent droplets passes directly into a desolvation chamber where the droplets are converted into solvent-free particles before reaching the exit nozzle. To aid faster evaporation of solvent molecules, the temperature of the chamber is maintained slightly above ambient. Momentum separation of the resultant stream of gas, solvent vapour and solute microparticles occurs between the desolvation chamber and the ion source. This is achieved by a series of skimmers placed in line with the nebulizer jet and exit nozzle. Differential pumping is effected in the regions between the skimmers. Expansion into the lower pressure regions leads to the formation of a high velocity jet of solute microparticles. Most of the helium and solvent vapours are removed in these lower pressure regions, leading to solute enrichment. The solid solute microparticles enter a conventional EI/CI ion source and are rapidly converted to the gas phase by flash vaporization upon contact with the heated walls of the source. Subsequent ionization by electron impact or chemical ionization follows.

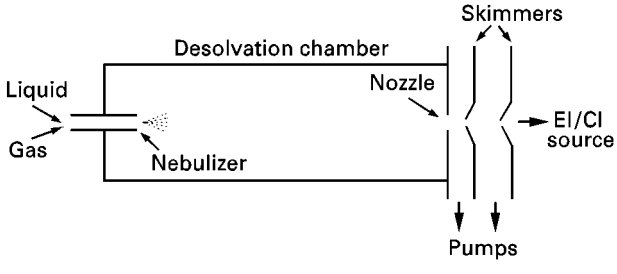

Figure 1 Simplified schematic of a particle beam interface.

The particle beam interface offers the advantage of producing library-searchable mass spectra but there are limitations of volatility and thermal stability for the analytes. In common with most LC interfaces for MS, the use of involatile buffers is best avoided, as is the use of mobile phases with a high water content. Disadvantages of the particle beam interface lie in the lack of sensitivity compared to other techniques which rely on 'soft ionization' methods, but careful optimization can lead to detection limits in the nanogram range for full scan acquisitions and use of selected ion monitoring can improve this to picograms. The development of particle beam interfaces capable of operating at lower flow rates would enable an increase in sensitivity to be achieved.

Continuous Flow–Fast Atom Bombardment

This technique relies on ionization of the sample by FAB. In a FAB ion source the sample is bombarded by a beam of energetic (usually 8 keV) atoms, resulting in the production of ions via the phenomenon of 'sputtering'. This process, although not fully understood, may be viewed as a series of impact cascades through the uppermost layers of the sample, resulting in the ejection of neutral and charged particles from the sample surface. Inert gases (Ar or Xe) are used to produce the atom beam but it is now more usual to employ a beam of fast ions, usually Cs^+, with energies up to 30 keV. This latter method is also referred to as liquid secondary ionization mass spectrometry (LSIMS).

The sample material is deposited on the end of a direct inlet probe along with a viscous liquid (matrix). The matrix is necessary to prolong sample lifetime as mass spectra produced from solid samples are transient. Ideally the liquid matrix (e.g. glycerol, m-nitrobenzyl alcohol) should be capable of dissolving the sample molecules, be inert and have a low

vapour pressure. The ion source is normally operated at ambient temperatures and it is therefore possible to obtain mass spectra from thermally labile and/or involatile materials. Compounds of RMM up to 2 kDa are routinely analysed by FAB and it is possible to obtain mass spectra from larger molecules. FAB mass spectra generally show abundant ions of the type $(M + H)^+/(M - H)^-$, thus allowing RMM information. These even-electron species are relatively stable and there is not always sufficient fragmentation to be structurally informative. Chemical noise is often a problem in FAB mass spectra as it may obscure low intensity fragment ions, and peaks arising from the matrix may coincide with sample peaks, hindering interpretation.

CF-FAB (other variations are known as dynamic FAB or frit-FAB) employs either FAB or LSIMS to effect ionization. All rely on the introduction of liquid through a capillary that terminates at the end of a direct insertion probe (**Figure 2**). Different designs of probe tip have been developed but all require an even spread of liquid over the surface which allows the production of a stable sample ion current. The solution entering the ion source requires the presence of a matrix liquid, normally in the range 1–10% by volume, and this is usually introduced post-column for LC-MS applications. This reduction of the sample/matrix ratio may provide improved signal to chemical noise ratios and peaks associated with the matrix may be either absent or of low intensity. A reduction in the sample suppression effects observed in FAB may also result.

This is a very simple interface design and is applicable to many thermally labile and/or polar samples. The probe tip does require heating (up to 60–70°C) to maintain evaporation and prevent freezing due to latent heat of vaporisation. The prime disadvantage is the restriction imposed on flow rates which are in the range 1–10 $\mu L\ min^{-1}$ and therefore a split in the column eluent is required for all but capillary columns. Splits may be achieved by a number of methods, involving the use of T-pieces, balanced columns and pneumatic splitters, either separately or in combination. Whatever method is employed, it should have low dead volume and provide a quick response to changes in solvent composition imposed by gradient elution.

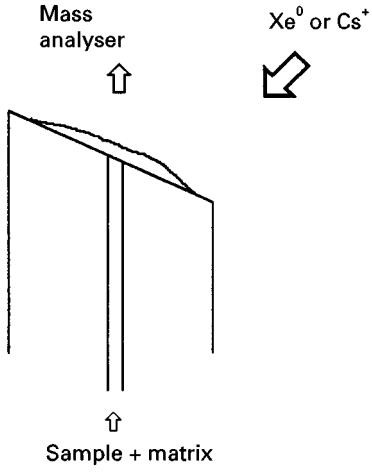

Figure 2 CF-FAB probe tip.

Thermospray

Thermospray ionization is effected directly from a sample solution and may be readily interfaced with HPLC. The thermospray ion source, which was developed from direct liquid introduction interfaces, can accommodate a wide range of liquid flows ($0.5–2.5\ mL\ min^{-1}$) but is limited to the use of volatile buffers. It is a soft ionization technique and produces mass spectra dominated by ions yielding RMM information, i.e. $(M + H)^+/(M - H)^-$, but modifications to the source have allowed a certain amount of controlled fragmentation to be induced.

The sample solution is carried into the source via a capillary tube which terminates in a heated block. This results in the formation of a supersonic jet of vapour which contains charged droplets, the charging of the droplets being aided by the presence of a volatile electrolyte (e.g. ammonium acetate). By a combination of ion evaporation and ion–molecule reactions, sample ions are formed and exit the source via a small sampling orifice. The excess solvent vapours are removed by a backing rotary pump. In those situations where it is not possible or desirable to add a volatile electrolyte to the mobile phase, ionization may be effected by a mechanism akin to CI. This is achieved either by use of an electron beam (often termed filament on) or by creating a plasma within the vapour-rich source, usually by a high voltage discharge from a needle – a technique sometimes referred to as a plasmaspray. A simple schematic of a thermospray source is shown in **Figure 3**.

A wide variety of compounds are amenable to thermospray but its ability to cope with large, nonvolatile molecules is poor and the mass range appears to be limited for routine use to compounds below ~1500 Da. The operating temperatures of the source must satisfy the requirement of efficiently vaporizing the sample but without thermal degradation. Positive and negative ions are often formed with equal facility but, in general, basic compounds are best studied in the positive ion mode: negative ion operation is more sensitive for acidic molecules. Lack of knowledge with respect to proton affinities often means that the best ionization mode needs to be determined experimentally.

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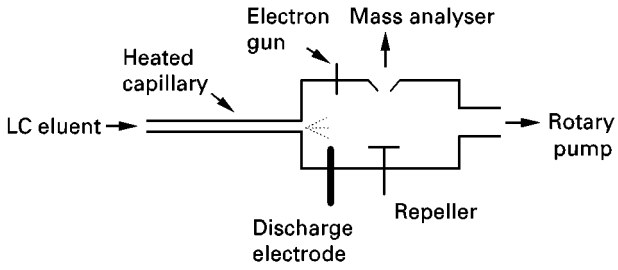

Figure 3 Simplified diagram of a thermospray ion source.

Lack of fragmentation is often observed in thermospray mass spectra but application of a higher voltage to a repeller electrode (located opposite the sampling cone) may be sufficient to induce the formation of fragment ions.

Criticisms of the technique have centred on the claims of poor reproducibility and compound dependence. The performance and optimization of the interface depend on the solution chemistry and solutions must be kept free of particulate matter that may lead to blockage of the capillary. Despite some disadvantages, many applications of thermospray have been described and it has been the mainstay for LC-MS development for a number of years. However, recent advances in alternative ionization methods may well see it superseded as the method of choice for LC-MS.

Atmospheric Pressure Ionization (API) Methods

In the sources so far described, ionization takes place in the vacuum region of the mass spectrometer, thus requiring removal, either through additional pumping or by a reduction in the flow rate of the mobile phase. The production of ions prior to entry to the MS high vacuum regions, i.e. at atmospheric pressure, would obviate these requirements. Development of atmospheric pressure ionization techniques has led to a rapid and exciting development in LC-MS instrumentation. Although API methods have been available for a number of years, it was not until the pioneering work of Fenn *et al*. that their potential was realized. The two variants normally employed in conjunction with HPLC are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).

Electrospray ionization ESI produces charged particles directly from solution at atmospheric pressure. Since its introduction in the mid-1980s it has developed into one of the most popular ionization techniques, especially for biomolecules. The source design is relatively simple and extraction of ions into the mass spectrometer is readily achieved. Although a variety of source designs have been developed and commercial instruments differ in this respect, the basic processes of ion information and extraction are similar.

In its simplest form, ESI is realized from a sample solution (flow rate 2–10 μL min^{-1}) introduced through a capillary into the ion source, which is at atmospheric pressure. The emerging liquid is formed into a fine spray of charged droplets by the presence of a potential difference of $\pm$ 3–5 kV applied between the capillary and a counter-electrode. The formation of gaseous ions from the sample solution occurs as a result of this droplet formation and subsequent desolvation. Formation of the charged droplets is reasonably well understood, but the process of ion formation from them is the subject of debate.

A typical source is shown in **Figure 4**.

The capillary delivering the liquid flow is contained, in a co-axial arrangement, within an outer stainless-steel capillary. A flow of gas through this outer capillary aids droplet formation and extends the usable flow rate up to $\sim$1.5 mL min^{-1}. Nitrogen is the usual nebulizing gas employed in this modification, sometimes referred to as ion spray. Beyond the counterelectrode is a sampling cone (or in some instruments this may be a short glass or steel capillary) which may be maintained at a low voltage ($\sim$30–250 V). Between this cone and the counterelectrode, a countercurrent flow of gas (usually nitrogen) is introduced. This gas, known as the drying or curtain gas, aids the desolvation process. Additionally, the source may be held at elevated temperatures ($\sim$60°C) as a further means of helping desolvation. Entry into the analyser region of the mass spectrometer proceeds via a skimmer held at ground potential. Stages of differential pumping (or cryopumping) reduce the source pressure (atmospheric) to that of the analyser ($\sim 10^{-5}$ mmHg).

Electrospray is an extremely soft ionization technique and results in the formation of ions representa-

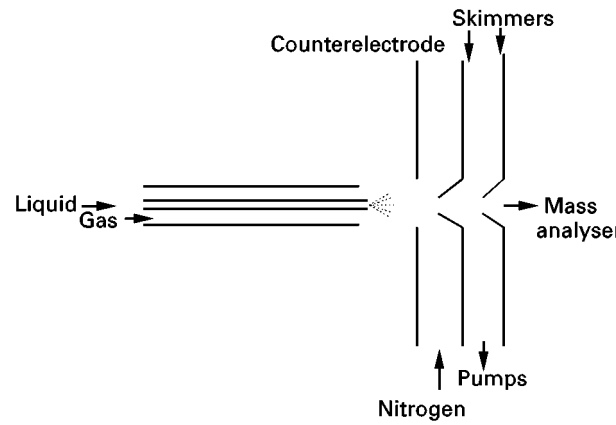

Figure 4 Ion source for electrospray ionization. For operation in APCI mode, a discharge needle would be placed between the inlet capillary and the counter-electrode.

tive of the intact molecule with virtually no fragmentation. For small molecules the mass spectra have a very simple appearance, generally showing just the protonated molecule ion and/or adduct ions, e.g. $(M + Na)^+$. The mass spectra of larger molecules, however, become more complicated because of the production of multiply charged ions. A series of molecule ions of the form $(M + nH^+)^{n+}$ is produced, where n varies according to the number of sites on the molecule which are able to accept a proton. The molecular mass of a compound is calculated from the ion series by a deconvolution algorithm contained in the instrument's software (though it can be done manually!). An example of a typical ESI spectrum and the result of deconvolution is shown in **Figure 5**. Formation of negative ions occurs in electrospray, with both singly and multiply charged species being formed. The choice of ionization mode depends on the proton affinities of the analytes.

In addition to molecular weight information, ESI mass spectra may contain ions representative of both specific and nonspecific interactions that are noncovalent in nature. Fragmentation of intact molecule ions may be induced within the source region by manipulation of the cone voltage. The application of a voltage (up to 250 V) between the counter-electrode and the first sampling cone will lead to low energy collisions between sample ions and the curtain gas. Depending on the energy of these collisions, declustering and then fragmentation may occur.

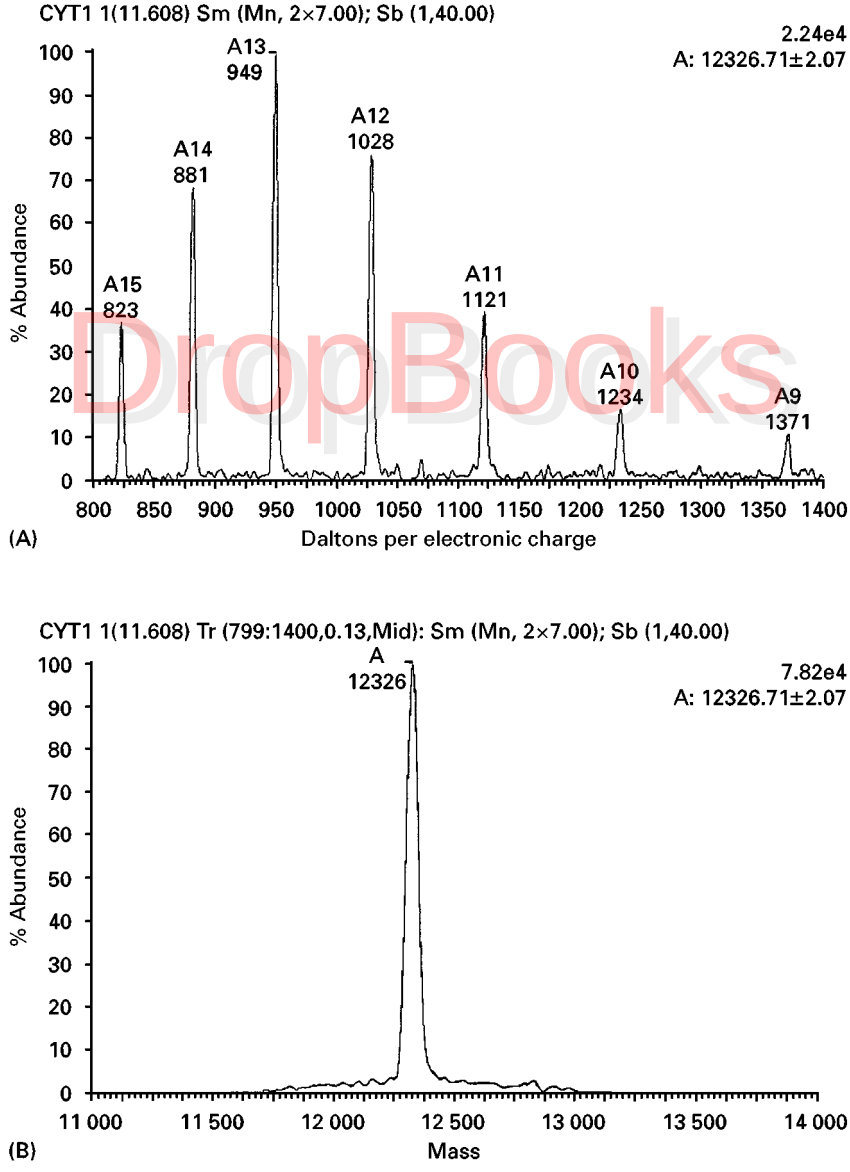

Figure 5 (A) ESI mass spectrum of cytochrome *c*; (B) deconvoluted mass spectrum of cytochrome *c*.

Since its introduction, ESI has undergone rapid development and has seen widespread application, especially in the biochemical field. Commercial instruments with dedicated ESI sources are readily available, ranging from simple benchtops to sophisticated tandem mass spectrometers.

The development of orthogonal sources has allowed the use of non-volatile buffers for LC-MS whereas 'in-line' sources are restricted to volatile buffers.

Atmospheric pressure chemical ionization An APCI source relies on the formation of reactant ions and their subsequent reaction with sample molecules. These reactant ions are formed at atmospheric pressure by a corona discharge achieved by maintaining a stainless-steel needle at a voltage of 3–6 kV. The source design for APCI, for LC-MS, is very similar to that of ESI, the major difference being the addition of the discharge needle in the region between inlet capillary and the counterelectrode. The LC eluent is converted into a fine droplet spray by a nebulizing gas and this is followed by vaporization in a heated region (up to 500°C, depending on the instrument) of the capillary. This rapid desolvation and vaporization minimizes any thermal decomposition. Chemical ionization of the sample is effected via ion molecule reactions: the reactant ions are formed from the LC mobile phase. Operation in either positive ion or negative ion mode is possible depending on the nature of the analyte. The use of a curtain gas aids declustering in a manner similar to electrospray. Molecular weight information is readily provided, but to obtain structural information the use of collision induced decomposition (CID) experiments is required.

Mobile phase flows from 0.1 to 2.0 mL min^{-1} can be accommodated, eliminating the need for splitting. Both volatile and, with the advent of orthogonal sources, nonvolatile buffers are tolerated and mobile-phase compositions of up to 100% water are permitted.

Mass Analysis

All the above ionization techniques may be used in conjunction with different types of mass analyser, though the use of single or multistage quadrupoles is most common. The reader is referred to the chapter on GC-MS for a discussion of sector and quadrupole analysers.

Ion traps, time-of-flight and ion cyclotron resonance mass spectrometers have all been used in LC-MS instruments: a full treatment of them is beyond the scope of this article.

The ion trap is a device in which ions may be stored and consecutive experiments carried out upon them, i.e. mass spectrometry in time rather than space. Ions may be produced directly in the trap, e.g. by EI (GC-MS) or they may be injected from an external source, e.g. ESI. Technological developments have ensured continued improvement to the mass range and resolution. The ability to undertake sequential CID experiments is a powerful feature of modern instruments. Dedicated LC-MS instruments employing ion traps in conjunction with ESI and APCI are now available.

Time-of-flight analysers are particularly well suited to pulsed ion sources, e.g. matrix-assisted laser desorption ionization (MALDI) and offer increased sensitivity over scanning instruments. Recently, pulsed orthogonal electrospray sources have been described in conjunction with time-of-flight.

The above method of mass analysis relies on the premise that, if ions of different masses leave the source at the same time, they will arrive at the detector separated in time. In practice, ions are accelerated into a field-free region (drift tube) with up to 20 keV energy. If all ions have the same kinetic energy then their velocity is dependent on their mass; thus, their time of arrival at the detector is different (light ions first). A pulse of ions is generated and the time taken for each ion to arrive at the detector is measured. Sophisticated electronics is required to measure these times accurately; in addition, a method of obtaining the start time is required. Ionization techniques capable of being pulsed are used, e.g. plasma desorption, laser desorption, atom/ion guns – these produce packets of ions with each pulse. Orthogonal instruments are now being developed which will allow the use of continuous ion sources such as electrospray (a simplified schematic is depicted in **Figure 6**). Traditionally, the resolution of time-of-flight analysers was considered to be low but modern instruments are capable of resolving powers up to 10 000. They have high transmission and are thus capable of high sensitivity; in addition they have a very high mass range. With the introduction of MALDI they have undergone a renaissance and continue to be developed. Commercial instruments interfaced to electrospray have recently been announced.

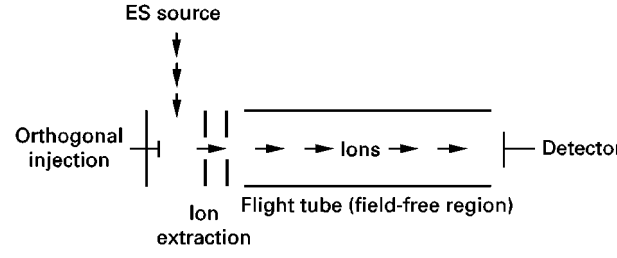

Figure 6 Schematic of a time-of-fight mass analyser with an orthogonal ESI source.

Instruments relying on ion cyclotron resonance (ICR) employ Fourier transform techniques and FT-ICR mass spectrometers are capable of achieving very high resolution. They require low pressures ($\sim$UHV) to operate effectively and the magnetic fields used are generated by super-conducting magnets. Ions, which may be formed directly within the ICR cell or injected from an external source (e.g. ESI, MALDI), are excited using a broad-band radiofrequency sweep and their cyclical motion induces an image current. This time domain signal is readily transformed to a mass spectrum by a Fourier transform. The very high resolution obtainable from this method of mass analysis enables separation of the isotopic peaks for each of the charge states resulting from ESI. This offers an advantage in the assignment of values of m and z in ESI mass spectra containing several masses.

Tandem Mass Spectrometry

Of the ionization techniques described, only the particle beam method produces sufficient fragmentation to give structural information. The softer ionization methods allow for RMM determination from either protonated molecule ions and/or adduct species, with little or no structural information being available owing to lack of fragmentation. It is possible to induce some degree of fragmentation in both thermospray and API sources by manipulating the source conditions. A more specific means of promoting fragmentation involves the use of sequential mass analysis, i.e. the isolation of a precursor ion followed by its interaction with a target gas to induce fragmentation by collisional activation, thus allowing a product ion mass spectrum to be recorded. This and other types of CID experiments can be carried out in time, in an ion storage device or in space using scanning or time-of-flight instruments. For a full discussion of the instruments and experiments possible, the reader is referred to the Further Reading section.

The most commonly used type of tandem mass spectrometer is the triple quadrupole, and benchtop instruments of this type with a dedicated LC interface are commercially available. They are relatively easy to use and offer a range of MS-MS scans. In addition to the product ion scan and the reaction monitoring scan (both MS1 and MS2 operate in selected ion mode), two other scan modes are available – precursor ion and constant neutral loss. In the former, MS1 is scanned whilst MS2 is set to pass a specific ion, thus yielding information as to the origins of a specific fragment ion. This may be employed to identify those components of a mixture which contain a common functional group, e.g. sulfonated compounds will typically fragment to give an ion at m/z 80 (SO_3^-) in negative ion mode; thus a precursor ion scan of this ion should be specific for the presence of sulfonates. Neutral loss scans involve scanning both MS1 and MS2, but with the respective mass ranges offset by the mass of the neutral species.

Conclusion

This article has attempted to review the present situation with regards to the interfacing of LC to MS. LC-MS is now developing into a mature technique and modern instrumentation allows for routine and robust operation. Further developments will undoubtedly take place, with API and PB techniques to the forefront. The introduction of cheaper and easier-to-use benchtop instruments will promote continued expansion in the applications of this extremely powerful analytical method.

See also: **III/Drugs and Metabolites:** Liquid Chromatography–Mass Spectrometry. **Pharmaceuticals:** Chromatographic Separations.

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